

# Entomopathogens: Ecological Manipulation of Natural Associations

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The control of insect pests with entomopathogens is unique, in that naturally occurring host-pathogen relations are manipulated to the benefit of man: protecting agricultural crops and forests or controlling insect vectors of disease. The isolation and identification of a virulent pathogen is the initial step in the development of a potential control agent. Production of the pathogen in adequate quantities must be possible either *in vivo* (insects) or *in vitro* (artificial medium). To insure usefulness, the pathogen must remain viable in the formulated form and after application in the field. Since inactivation rather than persistence is a problem, the pathogens must be formulated, protected, and applied to insure satisfactory pest control action. Studying the natural host-pathogen interactions will be necessary in order to manipulate the pathogen effectively, by introducing it at the most opportune time in the life cycle of the target pest. Generally, insect pathogens are more selective than conventional pesticides; this will limit their use and industrial development. Development, at least in part, by the public sector may be necessary and desirable. The most promising areas for the use of pathogens are in integrated pest management and in situations where pests have developed resistance to chemical control.

## Introduction

The entomopathogens are a unique group of pesticides, and it may be difficult to do justice to this subject in a relatively short paper. This difficulty becomes even more apparent if one considers the variations among the different entomopathogens: some are very specific, some less so; some act as infectious organisms, some act through their toxins; they cover the entire gamut of microbial forms, from viruses to bacteria to fungi to protozoans, even some rickettsial diseases of insects are known.

Faced with this multitude of aspects, it may be wise to consider the basic principles first. What are we doing when we are using an entomopathogen for pest control? A pathogen-host relation is in most instances based on an endemic situation which probably has evolved along with other developments in the ecology of the earth. A complete destruction of the host species would make the pathogen obsolete, and so we can view the host-pathogen situation as a survival of both partners in a

balanced state. Under stress situations in the host, this balanced state is upset, and the endemic situation changes to an epidemic one. Although we have observed this phenomenon in many cases, it is still difficult to precisely define the parameters which change endemic to epidemic states. Population density, food supply, availability of the pathogen, climatic conditions, for example, may all be singly or together responsible. The use of insect pathogens as pesticides, simply put, is the artificial induction of epidemics, and herein lies our major difficulty in their use.

The science of epidemiology is difficult enough when just two interacting organisms, the pathogen and the target host, are involved. In the area of insect pest control, we start usually with three components: the pathogen, the insect, and the host of the insect. In some instances, superimposed on this "basic" interchange, are predators and parasites of the insect, which may lose their food or host supply when the insect population is decimated by the pathogen and, in turn, may not be present at the time a resurgence of the insect population occurs, after the insect has overcome the pressure of the epidemic (1). In another situation, where a specific pathogen is used, the host insect may, in fact, be

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controlled adequately; but it may also leave a void in the total ecology of the host plant which will be taken over by other destructive insects which are not susceptible to the pathogen.

These are just a few examples of what might happen. They are probably not unique to the use of insect pathogens in pest control, but in using, or attempting to use, microbial control of pests, we have become more aware of these complex situations. The biological control scheme may have an additional educational benefit: it may pave the way in forcing us to look at the biology and epidemiology of the pest, an understanding which will be needed in integrated pest management.

The microbial control of insects and mites has been discussed in depth in a book of that title (2). In the following, we will not attempt to review the subject exhaustively. This paper should be regarded as an overview of the subject, directed to those scientists who have worked in the areas of conventional pest control as well as to those whose interest lies in the areas of ecology, epidemiology, and microbiology, pointing out to them the unique problem areas which exist in biological pest control. Hopefully their interest will be kindled.

## Isolation and Identification

Entomopathogens with a potential for pest control are in most instances isolated from field-collected diseased insects. The pathogens are often maintained by individual laboratories, but it has happened that pathogens have been lost, and therefore it would be a more desirable practice to store them with a national or international reference center. Such a center, sponsored by WHO, is maintained at Ohio State University. The isolation of a microorganism from a diseased insect is an initial lead that a potential for control exists. Before embarking into further development, it must be determined that the isolated microorganism was in fact the cause of death or whether it was only a fortuitous contamination (3).

The identification of a pathogen is the next important step. Proper identification of a pathogen is needed for evaluating its possible effects on non-target organisms, for quality control in production, for evaluation of possible changes (mutations) during prolonged production and use, and for bioassays of environmental levels. The identification of microorganism is difficult, but should be viewed as having the same importance as knowing the chemical formula and other chemical properties of a conventional pesticide.

For bacterial control agents, the problem of identification is fairly easily solved. Established taxonomic techniques, including biochemical tests and serological techniques, allow ready qualitative identification. *Bacillus thuringiensis* is a good example of how serotypes and strains were developed, identified, and maintained; for any other bacterial control agent, a similar approach should be used.

The identification of viral control agents of invertebrates has not progressed to the same level of sophistication to which we are accustomed in dealing with viruses attacking vertebrates. There are several reasons for this situation: tissue culture techniques which enhanced progress in vertebrate virology are not as well developed for invertebrate cell cultures; also insect viruses and especially the baculoviruses are complex structures which call for different approaches to identification. Since insect viruses are generally very host-specific, the host spectrum was traditionally used for identification, rather than biochemical, biophysical, and serological methods. These shortcomings have been realized in the recent past, and efforts are under way to provide an identification system for insect viruses which is on a par with the systems applied to vertebrate viruses (4). Many methods, such as serum neutralization, fluorescent antibody techniques, radioimmunoassays, electrophoretic assays, peptide mapping, and ultracentrifugation are available and are awaiting application to the field of invertebrate virology.

Fungal control agents of invertebrates pose no further problems with regard to identification than fungi in general. Especially with higher fungi, a possibility of genetic drift does exist, but these problems can be practically eliminated by maintaining standardized spore cultures. Toxins of fungi are the major source of concern as far as residues on food and in the environment are concerned. Therefore, in situations where the viable fungus is only incidental to the pesticidal action, it is best to consider the toxin in much the same way as one would consider any other chemical. Where the viable fungus has an integral role, proper consideration must be given to the biological aspects.

Protozoan control agents for insects are at the beginning of their development with respect to identification. In the case of protozoans, we have one excellent example. The malaria parasite, a most important microorganism relative to human health, is extremely well identified; research expenditure and efforts have been great, due to its importance for human welfare. The insect pathogens, on the other hand, considered to be of much less importance, have not prompted development of the same sophisticated system of identification. Ident-

tification is based largely on morphological observation of developmental forms. However, recently some problems have become apparent in regard to a *Nosema* species which has been identified in human tissue. There is no unequivocal test available to determine whether or not this species is different from the insect pathogens. (5).

## Production

### In Vivo

Many insect pathogens, notably the viruses, are produced *in vivo*; some bacterial agents, such as *Bacillus popilliae*, also can only be grown in living larvae. This has various disadvantages. (a) The rearing of insects is difficult or cumbersome. For some insects, such as gypsy moth and tussock moth, continuous laboratory rearing is impossible. (b) The living insect can have additional diseases, and the product therefore may be a mixture of indigenous pathogens and the one used for inoculation. Since the identification methods are not fully developed, it may be very difficult to determine that the pathogen produced consists, in fact, only of the progeny of the inoculum. (c) The product will contain insect fragments which, in some instances, may have to be removed. (d) Diseased and dead insects are prone to spoilage and, therefore, the product will invariably contain bacteria, which add further to the adulteration of the product.

### In Vitro

When complications arise, as mentioned above, the production of insect pathogens *in vitro* may become the method of choice. For saprophytic forms like *B. thuringiensis*, this is easily possible, although initially the determination of the best suitable medium posed some difficulties. Also, many fungi can be produced using common fermentation practices. Serious attempts are being made to produce viruses *in vitro* by tissue culture methods (6). These efforts are still in the developmental phase, the main problems being the cost of production, inability to scale up to large production and, most notably, the fact that virus production in tissue cultures may produce "defective" viruses (7). Hopefully, these problems will not prove to be insurmountable, since, with respect to purity and uniformity of product, tissue culture-produced virus would be far superior to the one produced in living insects; but we will have to see if considerations pertaining to economics and efficacy will prohibit the production of tissue culture-produced virus.

## Stability of Product

Since we are dealing with living organisms or, in some instances, biochemical products of these organisms (toxins), the problems related to stability are quite different from those of chemical pesticides. Factors such as humidity and especially temperature will have a far greater detrimental effect on the resistance of pathogens to chemical and physical degradation. In order to develop a practical pesticide, stability—notably biological stability—must be assured. A product which must be used within 2 weeks after fermentation or which loses activity at 25°C (80°F) obviously will not go very far as a useful and generally acceptable pesticide. It is reasonable to attempt to develop products which have a shelf life of at least 12 months. There is a wide range of factors affecting the stability, and they depend on the nature of the pathogen. A pathogen (spore or resting stage) which does not lose activity through drying (freeze-drying or spray-drying) has better storability than one which must be kept in a moist state and/or at temperatures other than the ambient temperature.

## Residues

### Persistence in the Environment

The question of residues, persistence, and accumulation in the environment is closely related to the problems of epidemiology discussed in the introduction. *A priori* the insect pathogens persist in nature; they fluctuate in concentration, depending on the developmental and disease phase of the insect and climatic changes. In most instances, we do not have enough quantitative data to predict what we empirically know, i.e., that in certain cycles, insect populations become diseased, collapse, and eventually regenerate. Although difficult to assess, these data are needed for two main reasons: (a) they will allow us to add the necessary imbalance to the natural cycles for our benefit (timely control of pests) and (b) they will give a measure of the levels of pathogens to which man and the environment are naturally exposed. If it can be shown that this "natural" exposure may be less after the use of pathogens, the concern about possible adverse effects would be minimized. As an example, the residue levels of virus on cabbage and in the soil of cabbage fields has been studied over several years. Although the virus persists in the soil over the winter months, it will not initiate disease in insects soon enough to prevent crop damage in the next

growing season. New virus must be applied in order to achieve timely control of pests (8–10). Using the cabbage plant as a model, it has also been shown that cabbage on the market shelf contains high virus levels from naturally infected insects (11). The situation in the cabbage fields may not be typical for other crops, but it shows that a comprehensive analysis of the insect–pathogen–crop ecology is needed in order to obtain satisfactory results.

## Persistence for Pesticidal Purposes

Any pesticide must have some persistence in order to be effective. Contact between the insecticide and the target insect must be made. For insect pathogens, this contact is even more critical, since in most instances the pathogen must be eaten to be effective. The exception are certain fungi which attack and destroy the insect by invasion through the cuticle. Some insect pathogens may have short half-lives of only 1–2 days when applied to crops (12). For effective control, this is only marginal. Many factors have been cited for this rapid inactivation under field conditions: elevated temperature, sterilizing light irradiation, or microenvironment on the leaf surface, including pH and unknown inactivators released by plants (13).

To sum up, the pathogens remain in the environment; they naturally incite disease in endemic and epidemic proportions. On the other hand many pathogens, when deliberately used for the control of pests, have a relatively short effectiveness. From these apparent contradictory observations, it can be concluded that the pesticidal use of pathogens will become feasible once we understand fully how nature manages insect populations by means of disease. Man should have an added advantage, in that he can protect and formulate the pathogen.

## Bioaccumulation

From the previous discussion, it is apparent that the term bioaccumulation, as used for chemical pesticides, does not apply to insect pathogens. The accumulation from continuous use and translocations in the food chain can be excluded. The bioaccumulation of a pathogen is a cyclical event, naturally or artificially induced. The amount of a pathogen present in any given ecosystem varies considerably. The levels after a naturally or artificially induced epidemic may be several orders of magnitude higher than the natural background level or the amount introduced for pest control (14). The high levels, however, do not persist, but disappear rapidly once the disease cycle has come to an end.

## Analytical Methodologies

The analysis of residue levels or environmental levels, in order to be meaningful, must be based on bioassays and/or determination of viable microorganisms. This is not always an easy task. The insect pathogen may not be easily distinguished from other similar microorganisms in the environment, for example. The most commonly used practice is to feed a certain amount of foliage to susceptible insects in the laboratory and determine the mortality index. Saprophytic microorganisms can also be isolated directly on artificial media. An interesting comparison of these two methods has been reported recently for a particular *B. thuringiensis* formulation, a microbial pesticide which is applied as viable spores but actually acts through a toxin contained in the spores. After 1 day the viable spore count was reduced to 20% of the original activity, whereas the insecticidal activity (toxin) still was 80% in a bioassay. This example shows that only the bioassay is a true measure of insecticidal activity in the environment. Such methods as pathogen isolation and particle counts can be used as assays only if their relationship to the actual biological activity is known (15).

## Use Patterns

The use patterns for insect pathogens vary greatly and are dependent on the insect to be controlled, as well as the desired effect; both of these aspects may, in fact, be interrelated. One example is the control of the spruce sawfly (*Diprion hercynia*), a pest introduced to the American continent from Europe. A virus accidentally introduced into the pest population has successfully controlled the insect for years. A similar success was achieved in the control of the pine sawfly (*Neodiprion sertifer*). These are extreme and, in a sense, unnatural examples, since a disease-free population was translocated and later controlled with the natural disease from other parts of the world. In most other instances, a repeated introduction of the pathogen will be necessary. For agricultural pests which have several reproductive cycles in one growing season, a multiple application each year will be needed; for other pests, such as forest defoliators, a single application per year should be sufficient and, in fact, may afford protection for several subsequent years.

The desired effect also determines the use pattern or the choice of pathogen. Generally, an infected insect will continue to feed on the crop. Control must therefore be initiated early, so that the pest is still in its early larval stages, causing less

damage, or the plant can recover from initial damage. An exception to this mode of action can be found with *B. thuringiensis*, since it acts primarily through its endotoxin, which paralyzes the pest and stops it from feeding. Bacterial proliferation may or may not eventually contribute to the insect's death. The most promising uses for insect pathogens are therefore those where we can accept some foliar damage and can afford to await the eventual and slow, but perhaps more lasting reduction of pest populations and associated losses. The situation in forests, grasslands and in agricultural crops such as soybeans and other feed and grain crops are examples. On the other hand, vegetable crops may not lend themselves as readily to microbial pest control practices, because marketability and minor evidence of pest activity play an important role in the market value of the crops.

Most pests for which microbial control is effective or promising fall under agricultural-forestry pests and, among those, predominantly under the lepidopterous insects. Pathogens, primarily fungi, bacteria and protozoans, are also known to affect other taxonomic groups and have been isolated from medically important insects, such as disease vectors. There may be several reasons for the predominance of agricultural pests, especially lepidopterous pests, believed to be accessible to microbial control: the control of agricultural pests on a short term basis is economically more important than the control of disease vectors; it is technically easier to control agricultural pests than disease vectors, which usually have a more ubiquitous habitat; pathogens usually affect the larval stage of insects, and lepidopterous larvae are some of the most destructive ones; and, related to this, the control, especially partial control, of larvae of disease vectors may not reduce the incidence of disease by very much. In other words, the development of microbial pesticides may not only depend on the actual potential but more so on other considerations, such as economics and expediency. This is also a self-fulfilling prophecy, resulting in more interest research, and funding to develop potentially useful pathogens for agricultural purposes, whereas the knowledge about pathogens in the area of medically important pests lags further behind.

Formulating microbial control agents is of major importance. Surfactants and diluents must be added as in the case of chemical pesticides, but these substances must be chosen in such a fashion that they do not inactivate the biological activity of the pathogen. In addition, new techniques which afford protection to the pathogen must be sought.

For this purpose, "light screens" and encapsulation have been tried, for example, in order to extend the short half-life of the pathogen. Some success has been reported, but formulation research still needs special attention (16).

## Selectivity

The selectivity of insect pathogens is one of their most attractive features. There are, again, considerable variations; *B. thuringiensis*, for example, has a relatively wide host range but mostly affects lepidopterous pests. Some nuclear polyhedrosis viruses are specific to single species of insects. *Nosema locustae* is effective against several species of grasshoppers, *B. popilliae* is pathogenic only to the Japanese beetle. These few examples illustrate the variability of host spectrums; however, it should be pointed out that the specificity of insect pathogens, even of the "broad spectrum" pathogens, is still much narrower than the one of chemical pesticides, which are toxic to insects as well as other forms of life, including vertebrates.

The specificity of microbial control agents, although desirable from a safety standpoint, also has certain drawbacks. The development of insecticides has become very expensive, and industry may have no incentive to develop a pesticide which controls only one or a very few species at best. Furthermore, in many agricultural situations, the crops are attacked by more than one species; there are so-called pest complexes involved in the destruction of crops. If a pesticide removes only one pest from the complex, crop protection will not be achieved successfully.

Because insect pathogens act slowly and some are species-specific and cannot control insect complexes, it has been suggested that they be used in combination with chemical pesticides; this is the currently accepted practice. The benefit appears to be that fewer chemicals per acre are needed; or, in many instances, a microbial pesticide extends the field life of a short-lived chemical. The suggested reason for this practice is that the insect becomes moribund from the action of the pathogen and thus will be more susceptible to the chemical. It would appear that a sequential application, pathogen first, chemical second, would be the logical solution. Combinations of pathogens as well have been tried; with the more specific viruses, this may be necessary to control several insect species in the same environment. The combination of pathogens and chemicals has been reported to be successful by researchers in Eastern European countries (17, 18).

## Summary and Conclusion

The use of microbial pest control agents has many potential advantages but also disadvantages, all of which are related to the central problems of epidemiology and natural association of the pathogen and the pest. Some of the advantages are that insect pathogens are more specific than chemical pesticides and thus have less effect on useful insects; no harmful effect on man, other vertebrates or the environment has been demonstrated (this safety aspect, however, needs further experimental confirmation for many pathogens); there is less of a possibility of developing resistance. The natural and long-term pest-pathogen association supports this hypothesis. Some of the disadvantages are that for effective use, more knowledge on the biology and interactions of pest and pathogen are needed than for chemical pesticides; the usually narrow host spectrum inhibits commercial development of the pathogen and may affect efficacy in complex pest situations; viability (shelf life, persistence of levels in the environment which control pests efficiently) is a problem.

A final and very important consideration is funding of development of microbial control agents. Many research dollars have been spent and much lip service has been given to point out the desirability of microbial pesticides. In contrast to this, only three microbial control agents are registered in the U.S., another half-dozen have been used on a local basis or are being developed, and another handful is being used or developed in Europe. If one chooses to be critical or pessimistic, it seems that the output lags far behind the apparent efforts. Is it not time in 1975 to balance the account? Is it not time now to direct our effort to register and use these pesticides, if they are in fact useful and safe? We suggest that the responsibility cannot be laid on only one doorstep, such as USDA, EPA, NIH, industry or university research. All share in the responsibility, and that is where the problem may lie. Industry did not have the responsibility to develop chemical pesticides, it had the financial incentive. For microbial insecticides we must also look for an incentive, obviously not a financial one, but maybe a moral one supported by the public sector. And we must refrain from procrastination, a usual attribute of moral commitments.

What is it then that we can do? Agricultural research (government, state, and private) must develop efficacious, stable and useful products, not simply water suspensions of pathogens. The industrial know-how must be solicited by collabora-

tion, contracts, and grants. EPA must provide guidance so that the efficacy and safety of the product can be demonstrated to its satisfaction. This guidance must be reasonable and to the point, taking into account all that is known about the action the environmental levels and limitations of insect pathogens. The U.S. Public Health Service should be approached to become involved in the assessment of public health effects of the insect pathogens and indicate, based on scientific considerations, whether such effects are likely to occur and how we could best monitor them.

The area of microbial pesticides offers a unique possibility to do prospective and prognostic research in the pesticide world. For baculoviruses, for example, a research, development, and registration program has been adopted by the University/EPA/USDA Coordinating Committee on Environmental Quality, Research, Monitoring, and Education, in order that research efforts on viral pesticides can be coordinated and the viruses can be developed and used safely as pesticides. Similar coordination programs may be envisaged for the timely development of other microbial pest control agents.

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